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Mitochondrial diversity analysis of *Glossina palpalis gambiensis* from Mali and Senegal

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Abstract

West African riverine tsetse populations of *Glossina palpalis gambiensis* Vanderplank (Diptera: Glossinidae) were investigated for gene flow, inferred from mitochondrial diversity in samples of 69 flies from Senegal and 303 flies from three river drainages in Mali. Four polymorphic mitochondrial loci were scored. Mean haplotype diversities were 0.30 in Mali and 0.18 over both Mali and Senegal. These diversities estimate the probabilities that two randomly chosen tsetse have different haplotypes. Substantial rates of gene flow were detected among flies sampled along tributaries belonging to the river basins of the Senegal, Niger, and Bani in Mali. There was virtually no gene flow between tsetse in Senegal and Mali. No seasonal effects on gene flow were detected. The implications of these preliminary findings for the implementation of area-wide integrated pest management (AW-IPM) programmes against riverine tsetse in West Africa are discussed.

Keywords

Glossina palpalis gambiensis; area-wide IPM; gallery forest; gene flow; riverine tsetse; single strand conformational polymorphisms; SSCPs; Mali; Senegal

Introduction

The tsetse *Glossina palpalis gambiensis* is one of the most important vectors of trypanosomes causing human sleeping sickness and trypanosomiasis in livestock across much of West Africa. Its range includes Senegal, Mali, Togo, Benin, Burkina Faso and Ghana (Jordan, 1993). It is replaced by the subspecies *G. palpalis palpalis* (Robineau-Desvoidy) in the south savanna-forest transition (Challier *et al.*, 1983). The mobility and tendency of tsetse flies to disperse ('vagility') is said to be great (Hargrove, 2003) and very often underestimated for tsetse species of the subgenus Nemorhina (i.e. the *palpalis* group), which inhabit gallery forests along West African river systems. Individually released *G. palpalis gambiensis* dispersed up to 21 km in 5 days along gallery forests in Burkina Faso (Cuisance *et al.*, 1985), whereas flies of the same species managed to disperse 8 km along rivers with almost bare banks (Challier, 1982). Linear dispersal of riverine tsetse occurs as much upstream as downstream but the rate of dispersal is influenced by factors such as host availability and season (Cuisance *et al.*, 1985).

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The paucity of data on movement and dispersal of riverine tsetse in West Africa is a serious obstacle to the development and planning of tsetse intervention campaigns. The ability of tsetse to move between patches of suitable habitat will determine its dispersal potential into areas where the target population is under suppression or has already been eliminated. Dispersal characteristics can be determined experimentally via mark, release and recapture experiments, but this is expensive, time consuming and uncertain. Genetic methods can be used instead as indirect measures of dispersal (Krafsur, 2003). The chief reason is that sub-populations or demes that exchange flies will be much more homogeneous genetically than demes that have not recently exchanged flies. Thus, the chief hypothesis to test is that gene frequencies among subpopulations will be homogeneous. Here we report testing of the foregoing hypothesis and its derivative hypotheses. The sampling was carried out in Mali and Senegal in 2002. Four locations were sampled in Senegal and a total of nine samples from three river drainages was sampled in Mali.

Methods

Biological material

A total of 372 flies were collected from three river drainages in Mali (n = 304) (the Niger, Bani and Senegal River Basins) and from the Niayes region in Senegal (n = 69) (Fig. 1). The Niayes, located north and east of Dakar, coincides with the extreme north-western limit of the distribution of *G. p. gambiensis* in Africa. The Niayes is characterized by vestiges of Guinean forest comprising oil palms (*Elaeis guineensis*) in low-lying areas where there are marshes or ponds from which water is sometimes available throughout the year. Favourable climatic conditions are found by *G. p. gambiensis* in these various types of vegetation, which are different from those typically known to the subspecies. (S. Leak, 2003, unpublished report to the IAEA).

DNA was extracted from the Mali samples at the Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory in Seibersdorf using the method of Bender *et al.* 1983. DNA was extracted from Senegal flies at Iowa State University using the CTAB method, as described earlier (Krafsur & Wohlford, 1999).

Mitochondrial variation was chosen because it is the most sensitive kind of selectively neutral genetic variation with which to study demographic phenomena. Mitochondrial genes are inherited as a single unit and there is no recombination. Mitochondrial variation was assessed using the single strand conformational polymorphism (SSCP) method. The advantage of SSCP is that it allows large samples to be processed, which is essential when studying demography. The procedures we used are detailed elsewhere (Krafsur & Wohlford, 1999; Marquez & Krafsur, 2002, 2003).

Primers and DNA amplification

The primers used in polymerase chain reactions (PCR) to amplify mitochondrial genes in *G. palpalis gambiensis* (Table 1) are described in Simon *et al.* 1994 and have been successfully used in other dipteran species including the mosquito *Anopheles gambiae*, the housefly *Musca domestica* and other tsetse: *Glossina morsitans s.l.* and *Glossina pallidipes*.

DNA sequencing

Thirty μL PCRs were carried as described in Marquez & Krafsur (2002, 2003). From each reaction 10 μL was used in SSCP gels and 20 μL kept for further DNA sequence work. The DNA was then sequenced from both the 3′ and 5′ ends on an ABI 377 sequencer.

Statistical considerations

SAS (SAS, 2001) and ARLEQUIN version 2 (Schneider *et al.*, 1997) software was used for the analysis of data. Mitochondrial diversity was analysed following the methods of Nei (1987) and Weir (1996). Nei's diversity index H_S, the average diversity over s populations, was estimated as

$$H_S = \sum h_e / s,$$

where $h_e = n(1 - \sum x^2_i /(n - 1))$ is the unbiased population diversity and x_i is the frequency of the ith haplotype. The minimum genetic distance between two populations was estimated as

$$D_{ij} = ((J_i + J_j) / 2) - J_{ij},$$

where $J_i = \sum pi^2$ is the identity of population i, and $J_{IJ} = \sum p_i\, p_j$ is the shared identity between populations i and j. Thus, D_{ij} represents the amount of identity unshared between populations. The average D_{ij} over s populations,

$$D_{ST} = \sum D_{ij} / s(s - 1),$$

is the diversity between populations and a measure of their genetic distance.

The total gene diversity, H_T, was estimated as the sum of the diversities within populations, H_S, between populations within subregions, D_{PS}, and diversity between subregions, D_{ST}, according to the relation

$$H_T = H_S + D_{PS} + D_{ST},$$

with

$$\mathrm{Var}(H_T) = \mathrm{Var}(H_S) + \mathrm{Var}(D_{ST}) + 2\mathrm{Cov}(H_S, D_{ST}).$$

Genetic differentiation between subregions was estimated as

$$G_{ST} = D_{ST} / H_T,$$

and that among populations within subregions as

$$G_{ps} = d_{ps} / h_t.$$

G_{ST} is analogous to Wright's F_{ST} and can be used to estimate rates of gene flow according to the island model

$$N_m \sim (1 - G_{ST}) / 2G_{ST},$$

where N_m is the mean number of reproducing females per generation. The variance of G_{ST} was estimated according to Chakraborty (1974).

Weir's analysis of variance of allele frequencies was performed using a nested design taking drainages and samples within drainages as random effects. The ANOVA afforded a hierarchical partition of allele frequency variance into its components and the estimation of the corresponding fixation indices F_{ST}, which can be interpreted as the correlation of haplotypes among population groups.

Results

In preliminary work, single strand conformation polymorphisms were evaluated at nine mitochondrial loci. Four polymorphic loci were chosen to study the population structure of *G. p. gambiensis: 16S2, CO1, CO2TL2* and *CyB1*. Two SSCP variants were detected at *16S2* and *CO1*, three variants at *CO2TL2* and five variants at *CyB1*. Because mitochondrial genes are inherited as a single unit, there was a total of nine variants or 'haplotypes'.

Veracity of SSCP

The nucleotide sequence analysis of SSCP electromorphs, or gel phenotypes, confirmed their authenticity (data not shown). In addition, the analysis showed that the electromorphs at the four loci each consisted of at least two nucleotide sequences (genotypes), thereby underestimating diversity. Resolution averaged over the four loci was 67%, estimated by the total number of gel phenotypes divided by the number of genotypes. The 'cost' of missing some variation is that genetic differentiation may be underestimated and gene flow consequently overestimated. The SSCP method, however, affords a cost-effective way of estimating mitochondrial variation in large samples.

Mitochondrial diversities

Haplotypes were determined for a total of 372 flies, 303 of which were sampled in Mali and 69 in Senegal. The nine haplotypes were distributed among the 13 *G. p. gambiensis* samples as shown in Appendix 1.

The most frequent haplotype, AAAA, was present in 66% of the samples. Seven of nine haplotypes were 'rare' in that each accounted for less than 5% of the total.

Subpopulation diversity estimates H_S varied among samples from 0 to 0.83 (Table 2). Diversity H_S is the probability that two randomly chosen flies have different haplotypes, and *Js* is the probability that they are the same. Senegal flies were monomorphic – there was no detectable diversity. Mean diversity among the Mali flies was 0.299. If the Senegal flies were included, overall mean diversity becomes 0.18.

Geographic considerations

The distribution of haplotypes by country is shown in Table 3. The Senegal haplotype *G. p. gambiensis* samples are arranged by river drainage in Table 4. Most flies (266) were sampled on the River Niger, and the fewest (19) on the River Bani. Only one of nine haplotypes was shared among river drainages. It seems that little gene flow occurred between *G. p. gambiensis* on the upper and lower reaches of the River Senegal because all flies from Senegal were haplotype BAAA and all flies in Mali captured on the River Senegal were of different haplotypes. The Senegal haplotype was present in less than 1% of the Mali flies.

Genetic differentiation of subpopulations

Analysis of molecular variance (Table 4) indicated a trivial amount of genetic differentiation (0.06%) between Mali subpopulations in river drainages.

If we include Senegal samples in a nested ANOVA, however, variance attributed to drainages increased to 50% and F_{ST} becomes a highly significant 0.68 (Table 4). Note that nearly 18% of the genetic variance was attributed to samples nested in river drainages, which translates to $F_{SD} = 0.36$. This is entirely due to the contrast between the Senegal samples and the Mali samples, all taken from the River Senegal and its tributaries. Senegal samples thus distort the overall picture of genetic differentiation and gene flow in *G. p. gambiensis*.

A quite different method of analysis is afforded by using Nei's (1987) methods (Table 5). G_{ST} is analogous to F_{ST}. According to the island model of dispersion, G_{ST} is equivalent to a mean exchange rate of 2.4 reproductive female flies per generation among the Mali river drainages. The mean exchange between Mali and Senegal is only 0.12, or one reproducing female fly every 8.3 generations.

Pairwise genetic distances are shown in Appendix 2. These are zero between subpopulations in the upper and lower River Senegal. Indeed, very high genetic distances were obtained between all Mali and Senegal sampled populations. Of rather more interest are the distances among the Mali samples. Siradobougou seems to be well differentiated from all other locations. We could predict that flies at the distal ends of tributary streams might show the most differentiation from each other by genetic drift. Flies near the confluence of tributary streams might be expected to show the least differentiation.

Seasonal effects on population structure

Mitochondrial variation in May and November samples was compared by ANOVA (data not shown). Only one drainage, the Niger, was replicated seasonally, which does not allow adequate evaluation of seasonal changes in gene diversity. The results show only a trivial variance, 0.003 of the total, between May and November haplotype frequencies. Only 4.7% of the variance was attributed to samples within seasons. F_{ST} for seasons was not significantly different from zero.

Discussion

Only nine haplotypes were detected in *G. p. gambiensis*. In contrast, the same methods demonstrated 32 haplotypes in G. pallidipes (Krafsur & Wohlford, 1999). The most frequent haplotype in *G. p. gambiensis*, AAAA, was present in 66% of the sampled flies. Of the nine haplotypes, six were rare, accounting for only 5.9% of the total. Thus, mitochondrial diversity was low in the sampled subpopulations and zero in the four samples taken in Senegal. The low diversity suggests severe reductions in population size sometime in the past, and little successful immigration from other regions where *G. p. gambiensis* has been more abundant.

Our findings indicate substantial gene flow among the tsetse populations of the different river basins in Mali. This might be related to the tendency of riverine species to spread out from the gallery forests into the surrounding savannas during the rainy season, with maximum dispersal when the evaporation is lowest and maximum contraction along the rivers when both temperature and evaporation are highest. The degree of spread seems to be positively correlated with the number of wet months but does not depend on the total rainfall (Nash, 1937). In that respect, *G. p. gambiensis* was able to disperse 1.5, 6.6 and 4.5 km through barriers of total deforestation (Cuisance *et al.*, 1981), across savannas and watersheds between gallery forests and through forest clearings (Glasgow & Duffy, 1947), respectively. Alternatively, tsetse may remain confined to the principle river systems and their tributaries, but dry season contraction of range may concentrate flies in linked refugia from which they disperse along seasonal streams with onset of the wet season. Such a pattern of dispersal could encourage genetic drift and this would be most evident in tsetse at the distal, upstream environments. We have no evidence of that, but Solano *et al.* 2002 obtained a significant Wahlund effect (F_{IS}) at two X-linked microsatellite loci in Burkina Faso *G. p. gambiensis*. Their interpretation was that dry season populations consisted of flies pooled from populations that had differentiated via genetic drift (Solano *et al.*, 2002). An alternative explanation is the presence of morphologically identical sibling species.

The foregoing observations are of prime importance in the context of creating sustainable tsetse-free zones in West Africa using an area-wide integrated pest management (AW-IPM)

approach, i.e. the management and elimination of *entire* tsetse populations, within a circumscribed area. Whereas available tsetse distribution maps, risk prediction models and population genetics data indicate fragmentation of the tsetse belt in East Africa (Krafsur, 2003), the distribution of riverine tsetse in the humid savannah area of West Africa seems more complex. Riverine tsetse species such as *G. p. gambiensis* seem to be restricted for most of the year to the riparian forests bordering the various river systems, and this close relationship between their spatial occupation of the habitat and hydrology/drainage systems could be exploited in AW-IPM intervention. It has been postulated that populations of riverine tsetse species might be completely confined to the rivers and tributaries of a specific basin because areas between adjacent basins prevent dispersion. According to this hypothesis, the 'primary river basin' could be considered and used as the 'geographical unit area of operation' in AW-IPM intervention campaigns, which would allow the creation of tsetse-free zones in West Africa. Our data seem to refute this hypothesis, although it needs to be emphasized that the sampling of flies was carried out only in a very small geographical area in Mali. More systematic tsetse sampling on a regional scale in West Africa is needed to allow a better assessment of the degree of isolation of the riverine tsetse residing in the various river basins. Data on the efficiency of the watersheds as barriers for the various river basins will be necessary to decide if tsetse intervention campaigns can be launched in West Africa according to the area-wide concept.

Mitochondrial data indicate that a large genetic distance separated *G. p. gambiensis* in Senegal from those in Mali, consistent with Solano *et al.*'s (1999) estimate, based on two X-linked microsatellite loci, of $F_{ST} = 0.18$ between Senegal and Burkina Faso. A simple explanation is that the Senegal tsetse population is descended from a small number of geographically isolated flies. Attempts to eradicate *G. p. gambiensis* from the Niayes region were made between 1970 and 1972 using ground spraying with dieldrin (Touré, 1973) and in the 1980s, when traps and insecticide impregnated targets were used in addition to the insecticide spraying (Diaite, undated). It was claimed that eradication had been achieved and reinvasion was deemed highly unlikely because the tsetse-infested region of the Niayes was considered to be well isolated from the main tsetse belt further to the south-east. *Glossinia p. gambiensis* was, however, detected in 1990 and its presence in the Niayes confirmed by surveys in 1997 (Diaite, undated) and 2003 (S. Leak, 2003, unpublished report to the IAEA). Claims of eradication of *G. p. gambiensis* from the Niayes region followed by subsequent reinvasions (Seye *et al.*, 2000) are unsupported by our genetic data, the monomorphism of which suggests resurgence from a small, relic population. Hargrove (2003) has shown the odds of detecting residual flies after a thorough control programme to be extremely long; moreover, he suggested that as few as 16 fertile flies may be able to re-establish a thriving population after control methods are relaxed.

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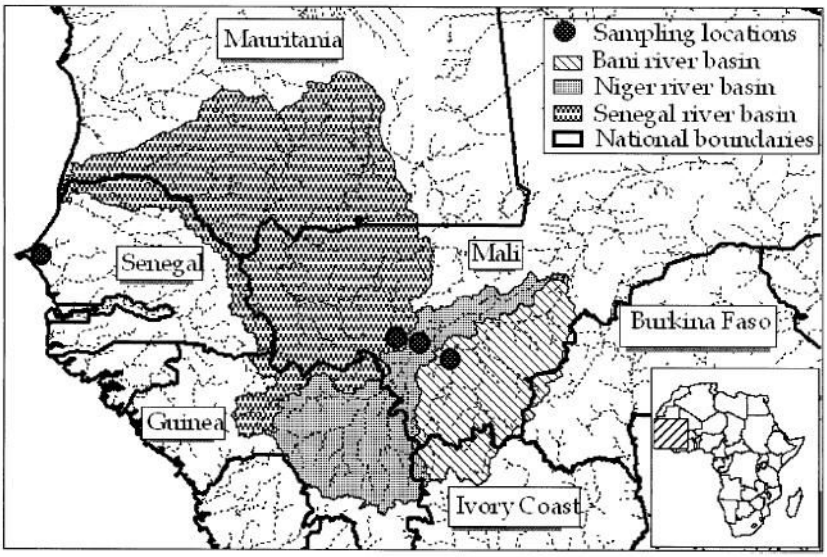

Fig. 1.
Map showing approximate sampling locations in Senegal and Mali.

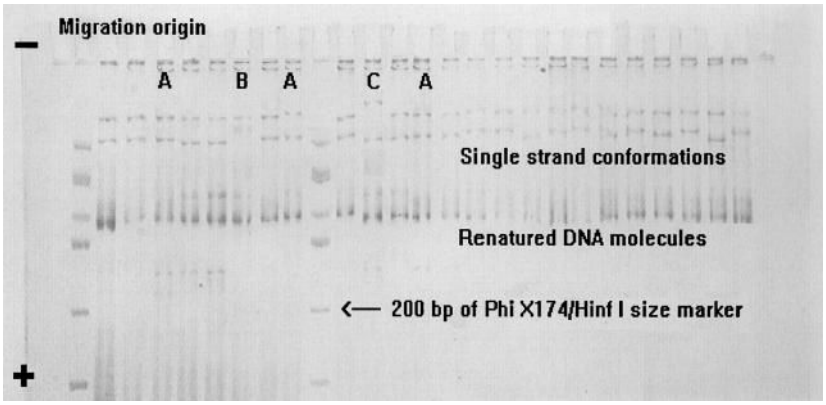

Fig. 2.
SSCP gel of CyB1 showing alleles A, B and C.

Table 1

Nucleotide primers used do demonstrate single strand conformational polymorphisms.

Locus	Sequence	Simon *et al.* 1994	Name
16S2	5′GGT CCC TTA CGA ATT TGA ATA TAT CCT3′	N1-J-12585	mtD-29
	5′ACA TGA TCT GAG TTC AAA CCG G3′	LR-N-12866	mtD-31
COI	5′GGA TCA CTG ATA TAG CAT TCC C3′	C1-J- 1751	mtD-7
	5′CCC GGT AAA ATT AAA ATA TAA ACT TC3′	C1-N-2191	mtD-9
Cy-B1	5′TAT GTA CTA CCA TGA GGA CAA ATA TC3′	CB-J-10933	mtD-26
	5′ATT ACA CCT CCT AAT TTA TTA GGA AT3′	CB-N-11367	mtD-28
COII-TLII	5′AAT ATG GCA GAT TAG TGC A3′	TL2-J-3034	mtD-13
	5′TCA TAA GTT CAG TAT CAT TG3′	C2-N-3389	mtD-15

Appendix 1

Haplotype frequency distributions by population sampled.

Population	BAAA	AAAA	AAAC	ABAA	AAAB	AABA	AACA	AAAE	BAAD
Dakar_PZ	1.000	—	—	—	—	—	—	—	—
Diak Sao	1.000	—	—	—	—	—	—	—	—
Pout	1.000	—	—	—	—	—	—	—	—
RD FAYA	—	0.721	0.256	0.023	—	—	—	—	—
RD KOBA	—	0.833	0.075	0.011	0.038	0.022	0.022	—	—
RD SOUSAN	—	0.947	—	0.053	—	—	—	—	—
RG DINA	—	1.000	—	—	—	—	—	—	—
RG DIO	—	1.000	—	—	—	—	—	—	—
RG KATI	—	1.000	—	—	—	—	—	—	—
RG Nougouriko.	—	0.900	0.100	—	—	—	—	—	—
RG Siradobougou.	—	0.500	—	0.250	—	—	—	0.250	—
RG Wayawanko	0.077	0.846	0.038	—	—	—	—	—	0.038
Sebikotane	1.000	—	—	—	—	—	—	—	—

Table 2

Mitochondrial haplotypes, identities (J_S) and diversities (H_S) by country of origin.

Country	Subpopulation	*N*	No. haplotypes	J_S	H_S ± SD
Mali	RD FAYA	43	3	0.59	0.42 ± 0.07
Mali	RD KOBA	186	6	0.70	0.30 ± 0.04
Mali	RD SOUSAN	19	2	0.90	0.11 ± 0.09
Mali	RG DINA	11	1	1.00	0
Mali	RG DIO	4	1	1.00	0
Mali	RG KATI	1	1	1.00	—
Mali	RG Nougouriko	10	2	0.82	0.20 ± 0.15
Mali	RG Siradobougou	4	3	0.38	0.83 ± 0.22
Mali	RG Wayawanko	26	4	0.72	0.29 ± 0.11
Totals and mean		303	9	—	0.27 ± 0.09
Senegal	Dakar PZ	12	1	1.00	0
Senegal	Diak Sao	18	1	1.00	0
Senegal	Pout	2	1	1.00	0
Senegal	Sebikotane	37	1	1.00	0
Totals and mean		69	1	1.00	0
Grand total and mean		372	9	0.82	0.18 ± 0.008

Appendix 2

Pairwise genetic distances among *Glossina palpalis gambiensis.*

	Mali								Senegal			
Sample	**Faya**	**Koba**	**Sousan**	**Dina**	**Dio**	**Nougour.**	**Siradob.**	**Wayaw.**	**Dakar**	**Diak**	**Pout**	**Sebik.**
Mali												
Faya	—											
Koba	0.0239	—										
Sousan	0.0588	0.0114	—									
Dina	0.0719	0.0180	0.0028	—								
Dio	0.0719	0.0180	0.0028	0	—							
Nougouriko	0.0284	0.0038	0.0075	0.0100	0.0100	—						
Siradobougou	0.1141	0.1194	0.1508	0. 1875	0. 1875	0.1475	—					
Wayawanko	0.0354	0.0057	0.0110	0.0163	0.0163	0.0070	0.1268	—				
Senegal												
Dakar	0.7929	0.8513	0.9501	1.0000	1.0000	0.9100	0.6875	0.7855	—			
Diak	0.7929	0.8513	0.9501	1.0000	1.0000	0.9100	0.6875	0.7855	0	—		
Pout	0.7929	0.8513	0.9501	1.0000	1.0000	0.9100	0.6875	0.7855	0	0	—	
Sebikotane	0.7929	0.8513	0.9501	1.0000	1.0000	0.9100	0.6875	0.7855	0	0	0	—

Genetic distance (the minimum genetic distance between two populations) = $2(Ji + Jj) - Jij$, where $Jij = \sum pipj$. $pipj$ is the shared identity for the same haplotype between samples *i* and *j*.

Table 3

Haplotype frequency distribution by country of origin.

Haplotype	Mali	Senegal	% of total	Total
AAAA	252	0	252	66.0
AAAB	7	0	7	2.0
AAAC	27	0	27	7.0
AAAE	1	0	1	0.2
AABA	4	0	4	0.8
AACA	4	0	4	0.8
ABAA	5	0	5	1.0
BAAA	2	69	71	19.0
BAAD	1	0	1	0.2
Total	303	69	372	100

Table 4

ANOVA on mitochondrial diversities among Mali *G. p. gambiensis* in countries and river drainages.

Source	d.f.	SS	MS	Exp_MS	Variance(%)	*F*
Mali only						
Between drainages	2	0.0678	0.0339	0	0.06	F_{st} = 0.0006
Within drainages	301	10.0074	0.0333	0.0332	99.94	
Total	303	10.0752	0.0672	0.0333		
Mali and Senegal						
Drainage	2	7.9163	3.9581	0.0420	50.35	F_{dt} = 0.503
Samples(drainage)	10	3.1004	0.3100	0.0148	17.69	F_{sd} = 0.356
Within samples	360	9.6095	0.0267	0.0267	31.96	F_{st} = 0.680
Total	372	20.6262	4.2949	0.0835		

Table 5

Hierarchical analysis of haplotype diversity.

Mali	
Diversity	
Within tributaries, H_S	0.239 ± 0.011
Between tributaries, D_{ST}	0.049 ± 0.001
Total, H_T	0.288 ± 0.100
Differentiation	
Between tributaries, G_{ST}	0.170 ± 0
Gene flow	
Between tributaries, Nm_T	2.44
Senegal and Mali	
Diversity	
Within countries, H_S	0.179 ± 0.008
Samples within countries, $D_{Sb(S)}$	0.023 ± 0.004
Between countries, D_{ST}	0.872 ± 0.173
Total, H_T	1.074 ± 0.362
Differentiation	
Samples within countries $G_{Sb(S)}$	0.021 ± 0.004
Between countries, G_{ST}	0.812 ± 0.102
Gene flow	
Subsamples in countries $Nm_{Sb(S)}$	23.00
Between countries, Nm_s	0.12